STUDIES ON THE MECHANISM OF ACTION OF IONIZING RADIATIONS. IV. EFFECT OF X-RAY IRRADIATION ON THE RESPIRATION OF SEA URCHIN SPERM

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It has been maintained by a number of investigators (see reviews by Scott, 1937; Fricke, 1934; Packard, 1931) that the respiration of single cells is strikingly resistant to the action of ionizing radiations, and this belief has been the basis for ignoring the role of enzyme inhibition when explaining the mechanism of action of ionizing radiations on living cells. In fact, Chesley (1934) reported that the respiration of sea urchin eggs, both fertilized and unfertilized, was unaffected by x-ray irradiation with as much as 43,000 r. Henshaw (1932, 1940) found delay in cleavage whether the eggs or the sperm were irradiated, but the smallest amount of irradiation used in these experiments was 4000 r. Evans et al. (1942) confirmed Henshaw's work on sea urchin sperm and reported that inhibition of fertilization by x-rays could be partially prevented by the addition of certain organic substances, as had previously been found by Dale (1940) when he discovered this protective effect against enzyme inhibition by x-rays. In previous reports it has been shown that the respiration of tissue slices from rats irradiated with doses of x-rays below 500 r was definitely inhibited (Barron, 1947) and that the respiration of grasshopper eggs was inhibited with doses of x-rays as low as 10 r (Tahmisian and Barron, 1946). It was considered important, in view of these last experiments, to reinvestigate the problem of the respiration of irradiated single cells. We present in this paper data on the effect of x-rays on the respiration of sea urchin (*Arbacia punctulata*) sperm.

Experimental

Sperm was obtained by cutting circularly the soft tissues of the sea urchin. By this process sperm was shed in small syracuse dishes. Sperm from several urchins was collected in graduated centrifuge tubes after filtration through gauze. Filtered sea water was added to fill up the centrifuge tube. Sperm was then separated by short centrifugation (10 minutes at 2000 r.p.m.), the supernatant fluid was discarded, and the remaining sperm was brought to the desired dilution starting with a stock dilution of 1 : 10 or 1 : 20. The stock suspension was thoroughly shaken and an aliquot was taken for dry weight. To obtain the dry weights, 0.5 cc. of this suspension was added to specially hardened pyrex tubes and was centrifuged in a Beams type air-driven high speed centrifuge with 85 lbs. air pressure for 10 minutes. The fluid was withdrawn and the tubes were dried overnight at 110°. The sperm dilution chosen for the irradiation experiments was 1 : 200. This sperm suspension (1.2 cc.) was pipetted into small glass vials of 15 mm. diameter and 20 mm. height.

These vials (20 for each set of experiments) were placed in an aluminum holder resting in a glass container full of cracked ice. X-ray irradiation was performed by Mr. Hyde of the Department of Radiology of the Marine Biological Laboratory. The x-ray machine operated at 182 Kv peak voltage and a current of 25 ma. through each tube. A filter of 0.2 mm. Cu was used. The measurement of respiration started 40 minutes after irradiation.

Effect of dilution on the respiration of sea urchin sperm

Gray found in 1928 that the respiration of sea urchin sperm increased on dilution, but no quantitative study of this phenomenon has yet been reported. Although Hayashi (1946) attempted to measure this dilution effect, his techniques of measurement of O_2 uptake and of dilution were faulty, and his paper gives no data but rough figures. It was, therefore, necessary to determine the optimum sperm dilution which would give the maximum Q_{O_2} values (c.mm. of O_2 uptake per mg. dry weight per hour), and steady rates of respiration for at least one hour. A large number of experiments were performed for this purpose with different sperm dilutions, from 1:10 to 1:1000. The O_2 uptake increased steadily up to a dilution of 1:200. It started to decline when the dilution was increased to 1:400. A dilution of 1:1000

TABLE I

The effect of dilution on the respiration of sea urchin sperm (Arbacia punctulata). *Values, Q_{O_2}, give c.mm. O_2 uptake per mg. dry weight per hour*

Dilution	Q_{O_2}
1:10	1.7
1:30	3.6
1:100	10.0
1:150	14.6
1:200	19.6
1:400	17.5
1:1,000	2.0
1:1,600	None

gave an O_2 uptake as small as that of sperm at a dilution of 1:10; furthermore, the respiration almost ceased at the end of one hour. When the sperm was diluted to 1:1600, there was no measurable respiration (Table I). This lack of respiration was not due to lack of sensitivity of the Warburg manometric technique, for when measurements were made with the very sensitive Cartesian diver technique of Linderstrom-Lang as modified by Claff [1] similar negative results were obtained. The increase in respiration of sperm with dilution is undoubtedly due to greater motility in the dilute solutions.

When experiments were performed on one sample of pooled sperm, the values agreed within 10 per cent. The experiments performed on successive days with different sperm suspensions and the same dilution did not give reproducible values. The average Q_{O_2} value of 29 separate experiments performed on separate days (each experiment in triplicate) with a sperm dilution of 1:200 was 19.6 ± 3.9, i.e., with 20 per cent variation. Sperm dilutions of 1:200 could be kept at 3° for six hours with little decrease in respiration (11 to 14 per cent).

[1] Personal communication.

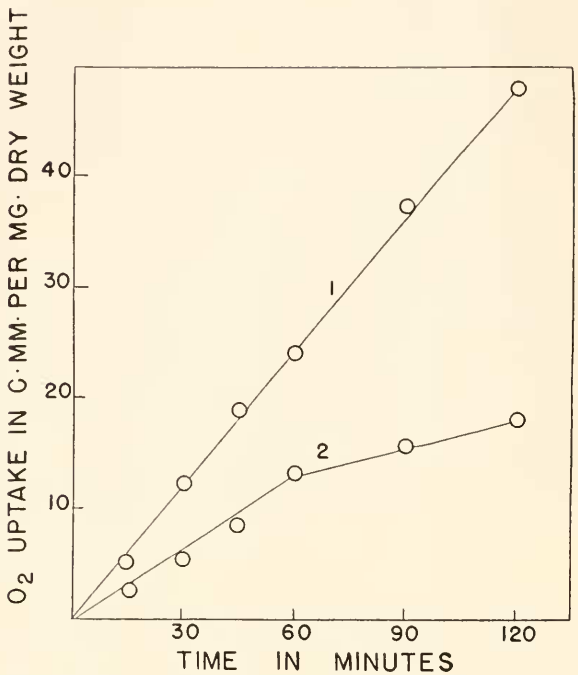

FIGURE 1. The effect of x-ray irradiation on the respiration of sea urchin sperm. Irradiation, 20,000 r. 1. Control; 2. Irradiated sperm.

It was not possible to obtain similar values in the O_2 uptake when the sperm was suspended in boiled sea water or in artificial sea water. The respiration of sperm in these cases was always much lower (20 to 40 per cent less).

Effect of x-rays on the respiration of sea urchin sperm

Once it was established that the optimum dilution for the measurement of sperm respiration was 1:200, all the experiments on the effect of x-ray irradiation were

TABLE II

Effect of x-rays on the respiration of sea urchin sperm. Dilution 1:200. Irradiation, 20,000 r. Figures give c.mm. O_2 uptake per mg. dry weight per hour

Exp. no.	Control	Irradiated	Inhibition (per cent)
1	14.8	6.0	59.4
2	16.2	3.8	76.6
3	12.0	5.0	58.4
4	23.9	8.9	62.8
5	18.3	5.3	71
6	13.1	2.8	79
7	29.6	17.6	54
8	18.6	10.6	43

performed with sperm suspensions so diluted. The sperm suspensions from 20 irradiated vials were collected in an Erlenmeyer flask from which 3 cc. were pipetted to each Warburg vessel. Thus, every experiment consisted of six control vessels and six vessels with irradiated sperm. Because of the reports in the literature on the resistance of respiration to x-ray irradiation, it was decided to start with 20,000 r. Such irradiation produced a marked inhibition of respiration, which increased in the second hour (Fig. 1). The degree of inhibition varied from sample to sample in all experiments. As an example of this variation the data of experiments of irradiation with 20,000 r are given in Table II. From this x-ray dose the amount of irradiation was diminished to 100 r with which an inhibition of 10 per cent was observed (Table III). The O_2 uptake inhibition on irradiation with 100 r did not increase in the second hour after irradiation (Fig. 2). In previous work

FIGURE 2. The effect of x-ray irradiation on the respiration of sea urchin sperm. Irradiation, 100 r. 1. Control; 2. Irradiated sperm.

TABLE III

The effect of x-rays on the respiration of sea urchin sperm. Sperm dilution 1:200. Q_{O_2} values, c.mm. O_2 uptake per mg. dry weight per hour

X-ray dose (r)	Q_{O_2} values		Inhibition (per cent)
	Control (c.mm.)	X-ray (c.mm.)	
20,000	22	7.5	66
10,000	19	13.3	30
1000	28	19.8	22
500	18	15.5	14
100	20	18	10

with grasshopper eggs irradiated with small doses of x-rays, there was inhibition of respiration when measurement of the O_2 uptake was made soon after irradiation, but a return to normal values five hours after irradiation (Tahmisian and Barron, 1947). Several attempts were made to see whether the respiration of sea urchin sperm inhibited by x-rays could also recover a few hours after irradiation. Soon after irradiation the sperm-containing vials were kept at 3° for five hours and the respiration was then measured. The degree of inhibition was the same as that obtained when measurements were made soon after irradiation.

The inhibition of respiration produced by x-ray doses between 1000 r and 100 r could not be attributed to H_2O_2 formation (even if there were H_2O_2 formation on irradiation of sea water) because small amounts of H_2O_2 increased respiration. Furthermore, a portion of this H_2O_2 would be destroyed by sperm catalase (Fig. 3). The catalase content of sea urchin sperm was such that 1 mg. dry weight would produce 33 c.mm. O_2 per hour at 25°, a figure which is eleven times less than the catalase content of mouse testicle. For these experiments sperm was washed three times in sea water, it was suspended in 10 volumes of water, and homogenized. Sperm thus treated gave no O_2 uptake.

TABLE IV

Effect of x-rays on the oxidation of succinate and acetate by sea urchin sperm. Substrate concentration, 0.01 M

X-ray dose (r)	Substrate	Q_{O_2} values		Inhibition (per cent)
		Control (c.mm.)	Irradiation (c.mm.)	
100	None	22.2	19.3	13
100	Succinate	30.0	22.8	24
100	None	20.5	18.0	12
100	Acetate	25.0	18.3	27
1000	None	20.5	15.0	26.9
1000	Succinate	27.2	20.0	38

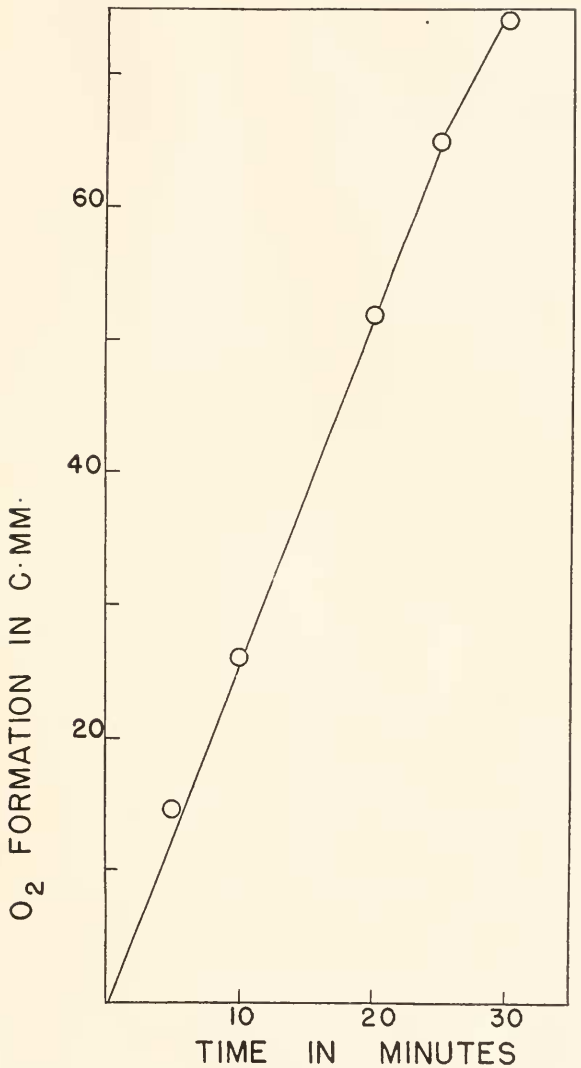

FIGURE 3. Catalase in sea urchin sperm. Buffer, phosphate, 0.01 M, pH 6.8; H_2O_2 0.01 M. Temperature 25°; Sperm, 0.1 cc. of 1: 10 suspension in H_2O, 1.8 mg. dry weight.

A number of intermediary metabolites are utilized by sea urchin sperm with an increase in the O_2 uptake. Of these, the utilization of succinate and of acetate (as measured by the increase of O_2 uptake) was tested after irradiation with 100 r. In both cases the inhibition of O_2 uptake was greater in the presence of substrates (Table IV), an indication that the enzymes for the oxidation of succinate and of acetate which belong to the group of sulfhydryl enzymes, are quite sensitive to the inhibiting effect of x-rays.

Summary

The respiration of sea urchin (*Arbacia punctulata*) sperm increased with dilution up to a dilution of 1 : 200, where maximum values were found. At this dilution the average Q_{O_2} value was 19.6 ± 3.9. When the dilution was increased to 1 : 1000 the respiration dropped sharply to 2.0. A dilution of 1 : 1600 gave no measurable respiration.

The respiration of dilute suspensions of sea urchin sperm (1 : 200) was inhibited by x-ray irradiation. A dose of 20,000 r produced an inhibition of 66 per cent which was further increased during the second hour; 10,000 r inhibited 30 per cent; 1000 r, 22 per cent; 500 r, 14 per cent; and 100 r, 10 per cent. When sperm was irradiated with 1000 r there was no recovery of respiration five hours after irradiation. Inhibition of respiration cannot be attributed to hypothetical H_2O_2 formation, for sperm suspensions contain catalase. The catalase value of sperm is 33 c.mm. O_2 formed by 1 mg. dry weight per hour, i.e., 3 micromoles H_2O_2 destroyed. On addition of succinate and of acetate to sperm irradiated by x-rays the O_2 uptake inhibition increased.

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